REMARKS

Claims 1-19 and 21-53 are pending in the application. Claims 33-51 and 53 are withdrawn from consideration. Claim 21 has been canceled without prejudice or disclaimer. Claims 1-5, 7-13, 15, 17-19, 23-29, 31, and 52 have been amended to better clarify what Applicants regard as the invention. Support for the amendments can be found throughout the specification, but particularly in original claims 15 and 31. New claims 54 and 55 have been added for consideration. Support for the new claims can be found throughout the specification and also in original claim numbers 11 and 27. No new matter has been added by way of this amendment. Thus, as a result of the foregoing amendment, claims 1-19, 22-32, 52 and 54-55 are under consideration. Reconsideration of this application is respectfully requested.

Rejection Under 35 U.S.C. §112

Claims 1-19 and 21-32 were rejected under 35 U.S.C. §112, second paragraph as being indefinite. In particular, the Examiner alleges that the claims are unclear for the following reasons:

The Examiner alleges that it is unclear what is meant by "a genetic construct having a single vector." A vector is a type of genetic construct, and as such the metes and bounds of the claim are unclear as are the other elements, (other than the vector), that it might contain.

The claims now recite a fusion polynucleotide comprising an immunoglobulin component selected from a group consisting of the kappa Ig light chain, the lambda Ig light chain, and an Ig heavy chain. The light chain, and heavy chain refer to protein, not nucleic acid, and as such, the Examiner alleges that the claim is confusing as it is unclear how the polynucleotide comprises the identified proteins. The same problem applies to the detectable protein, since it is unclear whether the applicant intends the claims to recite that the fusion polynucleotide is somehow complexed with the detectable protein or whether the fusion polynucleotide comprises a gene encoding a detectable protein. The Examiner suggests amending the claims to recite light chain and heavy chain genes, or a

gene encoding a detectable protein.

Furthermore, due to the claim amendments submitted with the response to the previous Office Action, the Examiner alleges that certain of the claims now lack antecedent basis for "at least one chimeric immunoglobulin gene" and "at least one detectable protein or peptide" because the claim now refers to fusion polynucleotides, not chimeric immunoglobulin genes, and further because the fusion polynucleotide comprises a detectable protein, not a detectable peptide. The Examiner further alleges that the difference in language renders the claims indefinite because it is unclear whether "the fusion polynucleotide "is the same as the "chimeric immunoglobulin gene". Claims 2-16, 18-19, and 21-32 depend on claims 1 and 17 and thus are included in this rejection.

Further, in regards to claim 17, the Examiner alleges that the claim is confusing in the recitation, "wherein said at least on detectable protein is present at the C-terminus of the gene product of said fusion polynucleotide with a flexible linker therebetween". The Examiner alleges that the fusion polynucleotide comprises an immunoglobulin component and a detectable protein, so the gene product of this fusion polynucleotide already comprises a detectable protein. Therefore, it is confusing where the flexible linker is supposed to be located. The Examiner has suggested that the claim be amended to recite that the flexible linker is located between the immunoglobulin component and the detectable protein.

In addition, the Examiner alleges that dependent claims 7-9 and 23-25 now lack antecedent basis for "an immunoglobulin molecule", since the previously amended claims 1 and 17 no longer refer to an immunoglobulin molecule.

In addition, claim 18 fails to further limit claim 17. Claim 17 already recites that the detectable protein is present at the C-terminus of the gene product of said fusion polynucleotide.

Applicants Amendments Based on the Above Rejection

- Regarding "a genetic construct having a single vector"

Applicants respectfully traverse the Examiner's rejection regarding the phrase "a genetic construct having a single vector; and have amended the claim where appropriate to delete

"a genetic construct". The amended claim now recites "a single vector".

- Regarding "...a fusion polynucleotide comprising an immunoglobulin component selected from a group consisting of the kappa Ig light chain, the lambda Ig light chain, and an Ig heavy chain..."

Applicants respectfully traverse the Examiner's rejection regarding the phrase "...a fusion polynucleotide comprising an immunoglobulin component selected from a group consisting of the kappa Ig light chain, the lambda Ig light chain, and an Ig heavy chain...", and have amended the claims where appropriate to recite "...said fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and a nucleic acid encoding at least two detectable proteins...".

- Regarding the lack of antecedent basis for "at least one chimeric immunoglobulin gene" and "at least one detectable protein or peptide"

Applicants respectfully traverse the Examiner's rejection regarding the lack of antecedent basis for the above-noted phrases and have amended the claims where appropriate to recite "fusion polynucleotide" and "detectable protein".

- Regarding the phrase "wherein said at least one detectable protein is present at the C-terminus of the gene product of said fusion polynucleotide with a flexible linker therebetween"

Applicants respectfully traverse the Examiner's rejection and have amended the claims where appropriate to recite that the flexible linker is located between the immunoglobulin component and the detectable proteins.

- Regarding the lack of antecedent basis for "an immunoglobulin molecule"

Applicants respectfully traverse the Examiner's rejection and have amended the claims where appropriate to recite "antibody", for which there is antecedent basis.

- Regarding the fact that claim 18 fails to further limit claim 17

Applicants respectfully traverse the Examiner's rejection and have amended claim 17 to delete the phrase regarding the detectable proteins being present at the C terminus of the gene product of the fusion polynucleotide. Thus, claim 18 now further limits claim 17, from which it depends.

Based on the foregoing amendments to the claims, withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Rejection Under 35 U.S.C. §103(a)

A. Fell et al. in view of Casey et al.

The Examiner has maintained her rejection of claims 17-19, 21-25 and 27-30 under 35 U.S.C. §103(a) as being unpatentable over Fell, *et al.* in view of Casey, *et al.*. More particularly, the Examiner asserts that claims 17-19, 21-25 and 27-30 are product by process claims and as such, patentability is based on the product itself. Thus, the Examiner alleges that even though Fell, *et al.* use a different vector system to modify the genome, as compared to Applicants' vector system, the ultimate product is the same.

Thus, the Examiner alleges that Fell, *et al*, is not required to teach making the cells using the exact method used by applicants as long as the cells made are the same. In the instant case, the Examiner alleges that Fell, *et al.* teach genetically modified antibody producing cells in which a component of an immunoglobulin gene has been replaced with a portion of the human variable or constant gene linked to an enzyme or substrate such as beta-galactosidase, alkaline phosphatase, or horseradish peroxidase (Fell, *et al.*, column 11, lines 24-66, and column 12, lines 1-12.) Furthermore, the Examiner alleges that Fell, *et al.* disclose that the antibodies produced by these cells are detectable and can be used as labeled antibodies in diagnostic assays without further modification (Fell, *et al.* column 11). In addition, the Examiner alleges that Fell, *et al.*, further teach that the replacement gene can be inserted into either or both of the light chain or heavy chain immunoglobulin genes (Fell, *et al.*, column 10). In addition, the Examiner alleges that

Fell, *et al.*teach that the replacement gene can be linked to the C-terminus of the chimeric immunoglobulin (Fell, *et al.*, Figures 1B + 1C). Furthermore, the Examiner alleges that Fell, *et al.* also teach a specific embodiment where the replacement gene encodes all or a portion of IgG1, such that the linked enzyme is present in exon G1 (Fell, *et al.*, column 14, lines 55-67). Thus, the Examiner alleges that Fell, *et al.*, teach cells with the same structural and functional limitations as the cells recited in the claims with the exception of the flexible linker. Casey, *et al*, has been cited for providing the teachings and motivation to use a flexible linker between the immunoglobulin gene and the detectable marker.

The Examiner further alleges that Casey, *et al.* teach the construction of detectable antibody by transfecting cells with a vector encoding a single chain antibody operably linked to a flexible glycine linker and GFP. Thus, since GFP is an autoflourescent protein, the Examiner alleges that it meets the claim limitations of claims 27-29. The Examiner further notes that while Casey, *et al.* use a bacterial system, which does not allow for glycosylation of the antibody, this teaching would be provided by Fell, *et al.*

Further, the Examiner alleges that Casey, *et al.* provides the motivation for substituting the flexible linker-GFP marker for the beta-galactosidase marker taught by Fell, et al. by teaching that flourescent labels provide high levels of sensitivity for a wide range of analytical assays (Casey, *et al.*, page 445). Thus, the Examiner alleges that the skilled artisan would have been motivated to substitute the nucleic acid sequence encoding the flexible linker-GFP taught by Casey, *et al.* for the nucleic acid sequence encoding the beta-galactosidase detectable marker in the construct taught by Fell, *et al.* based on the high level of sensitivity in detecting GFP and on the fact that flourescent antibodies can be directly detected without the need to treat the cells or purified antibodies with additional reagents such as Z-gal in the case of beta-galactosidase.

As the Examiner knows, in order to establish a proper *prima facie* case of obviousness, the Examiner must establish that there is a suggestion or motivation to modify the references or to combine the reference teachings; there must be a reasonable expectation of success; and the references or combination of references must teach or suggest all of the claim limitations (*see, e.g.,* MPEP § 2142). The teachings or

suggestions to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure (*In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cr. 1991)). The arguments advanced by the Examiner fail to meet all of these criteria for the current invention, as presently claimed.

The invention as claimed Claims 1 and 17 have been amended to recite a genetically-modified non-human mammal or a genetically-modified cell, respectively, containing a single vector comprising a fusion polynucleotide, said fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and a nucleic acid encoding at least two detectable proteins. The mammals and the cells secrete antibodies that are labeled with two detectable proteins, and the immunoglobulin component is separated from the detectable proteins through use of a linker molecule. The detectable proteins are selected from an autofluorescent protein, a visibly-detectable protein, an enzymatically active protein, a protein capable of interacting with another molecule to produce a detectable product, wherein said protein capable of interacting with another molecule to produce a detectable product is selected from the group consisting of an intein, a biotin-binding subunit of streptavidin or avidin, a His tag, a chitin-binding domain, or any combination thereof. The antibodies may be labeled with a combination of an autofluorescent protein and an enzymatically active protein.

Fell et al.

As noted previously, Fell et al teach genetically modified antibody producing cells which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both

the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

Fell et al. **do not teach or suggest** a genetically modified cell containing a single vector comprising a fusion polynucleotide, said fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and a nucleic acid encoding **at least two** detectable proteins. Furthermore, Fell et al. **do not teach or suggest** the use of a flexible peptide linker for separating the immunoglobulin portion of the molecule from the detectable protein portion of the molecule.

Applicants assert that **Fell et al. neither teach nor suggest preparation of a cell containing a fusion polynucleotide encoding an immunoglobulin gene and at least two detectable proteins.** Furthermore, Fell et al. **do not teach or suggest** that the antibodies produced *in vitro* should contain a flexible linker sequence between the immunoglobulin component and the at least two detectable proteins. Fell et al. **do not teach or suggest** the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al teach or suggest the possible variations of the detectable proteins envisioned by the present application, as claimed in currently amended claim number 17.

Casey et al.

Casey et al. describe the construction of a single chain antibody *in vitro* in a bacterial system. This antibody contains a flexible glycine linker and GFP.

As noted previously, Casey et al. do not teach or suggest the production of antibody producing cells *in vivo*, nor do Casey et al. teach or suggest the particular fusion polynucleotides or the antibody expressed which contains at least two detectable proteins now claimed in currently amended claim number 17. Furthermore, and as noted in the response to the previous Office Action, and as one skilled in the art can appreciate, the procedures for preparing an antibody molecule in a bacterial system do not allow for

correct post-translational modification of the antibody molecule, such as glycosylation, which is needed for maximizing function and specificity. Accordingly, Applicants assert that such differences between the prokaryotic and eukaryotic systems would not motivate one skilled in the art to combine the teachings of Casey et al. with those of Fell et al. Applicants assert that Casey took a single chain protein and showed that when this single chain protein is expressed as a fusion to GFP, it both folds and produces modest fluorescence. Applicants further assert that Casey used a single chain antibody that is radically different from a standard antibody which is made of two heavy chains and two light chains. The standard antibody requires a significant amount of disulfide bond formation and a considerable amount of folding. Thus, the standard antibody is constantly being monitored by the cell for proper folding and assembly. These antibodies are very sensitive to proper folding conditions and it has been shown many times that the standard antibodies cannot be made in bacteria. Thus, the antibodies of the present invention as currently claimed are not the same as the single chain molecule that Casey et al. used. Thus, Applicants assert that Casey et al. do not teach or suggest that an antibody as currently claimed can be synthesized as a fusion to GFP.

Furthermore, Casey et al. do not teach or suggest a genetically-modified immune cell containing a single vector comprising a fusion polynucleotide, said fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and a nucleic acid encoding at least two detectable proteins encoding an immunoglobulin component and **at least two** detectable proteins. Applicants again assert that one of skill in the art, upon review of the Casey reference, would not be motivated to combine these teachings with those of Fell et al. to proceed with the preparation of the fusion polynucleotides of the present invention due to the size and complexity of the fusion construct of the present invention as currently claimed. This assertion is based on the fact that Casey et al. use a **single chain antibody variable fragment** (scFv) for combining with **one (not two)** detectable label. Applicants assert that the teachings of Casey et al., when combined with the Fell et al. reference do not teach or suggest that the

larger immunoglobulin molecules prepared by the methods of the present invention could be made and maintain functionality. Applicants assert that there is simply no motivation or reasonable expectation of success for making the detectably labeled antibodies of the present invention based on the Fell et al. reference when combined with Casey et al, since the antibody molecules of the present invention containing two detectable protein markers are so radically different than the single chain antibodies of Casey et al.. In fact, Casey et al. discuss the potential problems with folding of the molecule in the proper configuration to generate a fully functional fluorescent scFv. This would lead one skilled in the art to believe that such problems would in fact be encountered even more so by using the full immunoglobulin gene fused to a gene encoding at least two detectable marker proteins, as taught by the present invention. Applicants assert that given these facts, one skilled in the art would not be motivated to combine the teachings of Casey et al. with that of Fell et al. to produce the fusion polynucleotides of the present invention.

The analysis under § 103(a). Neither Fell et al. nor Casey et al., alone or in combination, teach or suggest production of a fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component and a nucleic acid encoding **at least two detectable proteins**. Applicants assert that one of skill in the art would not be motivated to produce the fusion polynucleotides of the present invention as presently claimed for the following reasons. As noted above, Casey et al. utilize a prokaryotic system to generate a **single chain antibody fragment having one detectable label**. Casey et al. specifically state the difficulties of such an endeavor due to the possible loss of functionality in the detectably labeled single chain antibody fragment. Upon reading the authors statement regarding these potential problems, Applicants reiterate their assertion that one of skill in the art would not be motivated to attempt the preparation of an immune cell containing a fusion polynucleotide comprising a nucleic acid encoding a larger immunoglobulin molecule having two heavy chains and two light chains (not a fragment as taught by Casey et al.) and a nucleic acid encoding at least two detectable proteins because there would not be a reasonable expectation of success. Thus, while Fell et al. teach how to prepare an immortalized antibody producing cell using various

vectors to target the genes of interest, there would be no motivation to do so given the fact that there may be potential problems in folding of the proteins in an immunoglobulin molecule having two heavy chains and two light chains, while at the same time maintaining functionality of the protein components.

Accordingly, Applicants assert that there would be no motivation to combine the teachings of Casey et al. with that of Fell et al., which would then result in the particular aspects of the present invention, in particular, the cells containing the fusion polynucleotides encoding an immunoglobulin component and at least two detectable proteins, with the immunoglobulin component separated from the at least two detectable proteins by a flexible peptide linker, as currently claimed.

In light of the foregoing arguments and claim amendments, Applicants respectfully request withdrawal of this rejection.

B. Fell et al. in view of Casey et al. and further in view of Rajewsky et al.

The Examiner has also maintained her rejection of claims 1-9, 11-14 and 52 under 35 U.S.C. 103(a) as being unpatentable over Fell, *et al.* (U.S. patent No. 5,202,238) in view of Casey, *et al.* (June 2000) Prot Engineer. Vol. 13(6): 445-452 and Rajewsky, *et al* (U.S. Patent No. 6,570,061).

The Examiner alleges that Fell, *et al.* in view of Casey, *et al.* were already cited for providing the teachings and motivation to make genetically modified cells capable of expressing detectably labeled antibodies comprising various detectable markers including enzymatically active marker proteins and flourescent marker proteins. Further, the Examiner alleges that Rajewsky, *et al.* teach methods of making transgenic mammals comprising genetically modified chimeric immunoglobulins and provides specific motivation for producing chimeric antibodies *in vivo* over *in vitro* methods based on the drawbacks to *in vitro* methods of antibody production including the cumbersome work required to generate specific monoclonal antibodies of appropriate biological function and the difficulty in producing large quantities of these antibodies (Rajewsky, *et al.*, column 1). Rajewsky, *et al.* teaches that the use of transgenic mice overcomes these obstacles since every cell possesses the inserted replacement gene such that exposure to

different antigens will produce chimeric antigen-specific antibodies in quantities substantially larger that the amount capable of being expressed by cells in tissue culture. The Examiner alleges that it would have been *prima facie* obvious to the skilled artisan at the time of filing to use the homologous recombination vector taught by Fell, *et al.* to produce transgenic mice according to the methodology taught by Rajewsky, *et al.*.

The Examiner further notes that in regards to applicant's arguments regarding the insertion of two different detectable markers, such as a flourescent marker and an enzymatic marker, it is noted that claims directed to the insertion of two different detectable marker genes have not been included in this rejection.

The invention as claimed As noted above, claims 1 and 17 have been amended to recite a genetically-modified non-human mammal or a genetically-modified cell, respectively, containing a single vector comprising a fusion polynucleotide, said fusion polynucleotide comprising a nucleic acid encoding an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and a nucleic acid encoding at least **two** detectable proteins. The mammals and the cells secrete antibodies that are labeled with two detectable proteins, and the immunoglobulin component is separated from the detectable proteins through use of a linker molecule. The detectable proteins are selected from an autofluorescent protein, a visibly-detectable protein, an enzymatically active protein, a protein capable of interacting with another molecule to produce a detectable product, wherein said protein capable of interacting with another molecule to produce a detectable product is selected from the group consisting of an intein, a biotin-binding subunit of streptavidin or avidin, a His tag, a chitin-binding domain, or any combination thereof. The antibodies may be labeled with a combination of an autofluorescent protein and an enzymatically active protein.

Fell et al. As noted above, Fell et al teach genetically modified antibody producing cells, which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked

to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

Fell et al. **do not teach or suggest** the preparation of a fusion polynucleotide comprising an immunoglobulin gene and **at least two detectable proteins** for insertion into non-human mammals using the methods described in the present application. Furthermore, Fell et al. **do not teach or suggest** that the antibodies produced by said non-human mammal should contain a flexible linker sequence between the immunoglobulin component and at least two detectable proteins. Fell et al. **do not teach or suggest** the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al teach or suggest the possible variations of the detectable proteins envisioned and currently claimed in the present application.

Casey et al. As noted above, Casey et al. describe the construction of a single chain antibody *in vitro* in a bacterial system. This antibody contains a flexible glycine linker and GFP.

As previously discussed, Casey et al. **do not teach or suggest** the preparation of a fusion polynucleotide encoding a nucleic acid encoding an immunoglobulin component and at least two detectable proteins for insertion into non-human mammals using the methods described in the present application. Furthermore, Casey et al. do not teach or suggest use of a ***single*** targeting vector containing the immunoglobulin region and the detectable protein region construct, that is, a ***fusion polynucleotide*** for insertion into a cell of said non-human mammal. Furthermore, as one skilled in the art can appreciate, the procedures for preparing an antibody molecule in the bacterial system described by Casey et al. does not allow for correct post-translational modification of the antibody molecule, such as glycosylation, which is needed for maximizing function and

specificity. Such glycosylation patterns would on the other hand be accomplished using the genetically engineered non-human mammals of the present invention.

Applicants assert that Casey took a single chain protein and showed that when this single chain protein is expressed as a fusion to GFP, it both folds and produces modest fluorescence. Applicants further assert that Casey used a single chain antibody that is radically different from a standard antibody which is made of two heavy chains and two light chains. The standard antibody requires a significant amount of disulfide bond formation and a considerable amount of folding. Thus, the standard antibody is constantly being monitored by the cell for proper folding and assembly. These antibodies are very sensitive to proper folding conditions and it has been shown many times that the standard antibodies cannot be made in bacteria. Thus, the antibodies of the present invention as currently claimed are not the same as the single chain molecule that Casey et al. used. Thus, Applicants assert that Casey et al. do not teach or suggest that an antibody as currently claimed can be synthesized as a fusion to GFP.

Furthermore, while Casey et al. alone do not teach or suggest preparation of fusion polynucleotides in non-human mammals containing a large immunoglobulin gene and a detectable protein, the Examiner alleges that these teachings are provided by Fell et al. and Rajewsky et al. Applicants respectfully traverse the Examiner's allegations and assert that there is no suggestion or motivation to proceed with the preparation of the fusion polynucleotides of the present invention due to the size and complexity of the fusion construct for insertion into a cell of a non-human mammal, such as the blastocysts of the non-human mammals of the present invention. This is based on Applicants assertion that, Casey et al. use a **single chain antibody variable fragment** (scFv) for combining with a single detectable label. Applicants assert that the teachings of Casey et al. would not motivate or suggest to one skilled in the art that the larger and more complex immunoglobulin molecules having **at least two** detectable labels and prepared by the methods of the present invention could be effective or fully functional. In fact, Casey et al. discuss the potential problems with folding of the molecule in the proper configuration to generate a fully functional fluorescent scFv. This would lead one skilled in the art to believe that such problems would in fact be encountered even more so by

using the full immunoglobulin gene fused to a gene encoding **at least two** detectable marker proteins, as taught by the present invention, before insertion of such fusion polynucleotide into the non-human mammal.

Rajewsky et al. (U.S. patent No. 6,570,061). Rajewsky et al. teach the use of homologous recombination to replace the constant region genes of the murine immunoglobulin heavy or light chain with human genes in murine embryonic stem cells and the use of these cells to make transgenic mice which produce the chimeric antibody.

Rajewsky et al do not teach the introduction of at least two detectable marker proteins into the immunoglobulin molecule as disclosed in the instant application, and as currently claimed. Nor do Rajewsky et al. teach an *in vivo* method of generating detectable labeled antibodies specific for a preselected antigen. In particular, Rajewsky et al. do not envision the potential for combining an enzyme label with a fluorescent label on the chimeric immunoglobulin molecule such that the antibodies so produced would serve multiple functions for research or diagnostic use.

The analysis under § 103(a).

1. The references even when combined do not teach the present invention.

Applicants assert that the teachings of Fell et al., when combined with the teachings of Casey et al., and further when combined with Rajewsky et al, do not teach or suggest the fusion polynucleotides of the present invention that comprise a nucleic acid that encodes an immunoglobulin component and a nucleic acid that encodes at least two detectable proteins, which are separated by a flexible peptide linker. Applicants further assert that one of skill in the art would not be motivated to combine the teachings of these references to produce the genetically engineered non-human mammals of the present invention as presently claimed for the following reasons.

These references do not teach or suggest the *in vivo* production of genetically modified non-human mammals that produce the chimeric antibodies having the characteristics described and claimed in the instant application, in particular, at least two detectable protein markers. Applicants assert that one skilled in the art would not be

motivated to combine the teachings of Casey et al. with that of Fell et al. and Rajewsky et al. to produce the fusion polynucleotides of the present invention for insertion into the blastocysts of a non-human mammal. It was Applicants' own investigative work, which identified the need for production of a genetically engineered non-human mammal which would produce detectably labeled antibodies in response to antigenic challenge *in vivo*. Furthermore, Applicants' own work recognized the benefits of creating a chimeric molecule which was engineered to have a variety of functions retained such as the ability to bind antigen, the ability to fluoresce such that the antibody can be used for diagnostic purposes, and the ability to secrete the labeled antibody without interfering with the membrane bound form of the antibody, which may inhibit targeting or processing of the protein. In addition, the Applicants of the present application recognized that one can use the genetically engineered non-human mammals repeatedly and with various antigenic challenges. It is Applicants assertion that none of the foregoing would be obvious by combining the cited references.

In addition, Applicants respectfully draw the Examiner's attention to the specification on page 11, lines 14-22, continuing on to page 12, lines 1-4; and to page 15, lines 17-21, continuing on to page 16, lines 1-11, which provide support for how the Applicants envision the insertion of the genes for the detectable protein markers into the immunoglobulin gene, thus providing a chimeric antibody molecule that contains at least two detectable protein markers. More particularly, as noted in the specification on page 18, lines 9-17, one advantage of the present invention over the art cited is that a fusion polynucleotide that encodes a chimeric antibody may be prepared which may contain two different means of detection, for example, both a fluorescent marker as well as an enzyme marker, thus enabling the use of the antigen specific antibody for performing more than one function and thus may be applicable to various research and diagnostic applications, eg. the same chimeric antibody when generated with both a fluorescent label as well as an enzyme label may be used for immunohistological labeling of tissue sections, or for use for Western blot analysis or with a fluorescence activated cell sorter, or the same chimeric antibody when generated with both a fluorescent label and an intein that can be conjugated to a toxin or radiolabel can be used for tracking and therapeutic purposes. It

is Applicants assertion that none of the foregoing would be obvious by combining the cited references.

2. Casey et al. teach away from the present invention.

As noted previously, Applicants request that the Examiner's attention be drawn to certain statements by Casey et al, for example, on page 1, the third paragraph under the Introduction, whereby the authors attest to the potential problems with proper folding of the detectably labeled single chain antibody variable fragment to retain proper functionality. Casey et al. state:

"The design of an EGFP-scFv chimera posed some interesting questions regarding protein folding."

Applicants assert that the construct of Casey et al. is expressed in a prokaryotic organism and that one of skill in the art would not be motivated to combine the teachings of Casey et al. with those of Fell et al. and Rajewsky et al., since there would be very little expectation for success given the potential folding problems that occur with such single chain (scFv) molecules, let alone a larger construct comprising a full length heavy or light chain immunoglobulin molecule fused to at least two marker proteins. The task of making a genetically engineered non-human mammal whose genome has been altered such that the immune cells of said mammal secrete a detectably labeled fusion polypeptide (antibody) that is capable of proper folding and thus maintaining the specific binding characteristics, in addition to being labeled with at least two marker proteins, would seem daunting given the statements by Casey et al. This is particularly true given the larger size of the chimeric antibodies taught by Fell et al. and Rajewsky et al. These teachings, when combined, would lead one skilled in the art to believe that there would be very little chance of successfully generating a genetically engineered non-human mammal whose immune cells would be capable of secreting antibody molecules which retain their specific binding characteristics and functionality, in addition to being labeled with at least two marker proteins.

It was not until Applicants' present invention that an *in vivo* method for the production of genetically engineered non-human mammals that produce a chimeric

antibody containing at least two detectable proteins in response to a preselected antigen was possible. Furthermore, it was not until the present invention that genetically engineered non-human mammals or cells were produced that could respond to a single or multiple (different) antigenic challenge(s) and respond by secreting antibodies labeled with at least two detectable proteins following such antigenic challenge(s). In addition, there have been no teachings prior to the present invention, which demonstrated that mammals or cells could be engineered to produce antibodies labeled with either two detectable labels or a plurality of labels. The advantages of such an animal or cell were not apparent until the time of the present invention. It was only through Applicants' work whereby it could be appreciated that a non-human mammal or cell derived from said mammal could be used repeatedly for multiple and different antigenic challenges/immunizations and each time respond by producing a specific antibody that has at least two labels or multiple labels, and thus could be used concomitantly for different purposes, for example, for ease of purification of the antibody or for detection of antigen in tissue specimens. Based on the difficulties apparent to those skilled in the art prior to the time of the present invention for producing such a genetic construct, no one could have envisioned that such construct could result in expression of an antibody that not only maintains its specific binding characteristics, but also retains its two or multiple labels for detection of antigen in tissue samples or for purification purposes etc., thus resulting in an antibody that provides multiple functions.

Based on the foregoing arguments and claim amendments, withdrawal of the rejection is respectfully requested.

Fees

A check in the amount of $280 is enclosed to cover the 4 new dependent claims as well as the surcharge for multiple dependent claims. No other fees are believed to be necessitated by the foregoing amendment and response. However, if this is in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or credit any overages.

Conclusion

Applicants believe that the foregoing amendments to the claims and arguments place the application in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,

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